

STATE OF MAINE  
DEPARTMENT OF ENVIROMENTAL PROTECTION  
MAINE POLLUTANT DISCHARGE ELIMINATION SYSTEM  
GENERAL PERMIT FOR ~~FINFISH~~ ATLANTIC SALMON AQUACULTURE

MEG130000

Issued pursuant to  
38 MRSA section 413(10)  
Chapter 529 Code of Maine Regulations  
and  
Federal Water Pollution Control Act, 33 USC, section 1251, et seq.

This General Permit authorizes persons qualifying for and obtaining coverage under it to discharge to designated waters of the State pollutants resulting from the operation and maintenance of an ~~finfish~~ Atlantic salmon aquaculture facility in compliance with the terms and conditions set forth herein.

Area of Coverage: All marine waters of the State located East of Naskeag Point in Brooklin, except those waters in the area North of a line from Schoodic Point in Winter Harbor to Baker Island in Cranberry Isles, then West to Naskeag Point in Brooklin.

Receiving water classifications: Class SB and Class SC

This General Permit is issued for a term of five years from the date of signature.

DONE AND DATED AT AUGUSTA, MAINE, THIS \_\_\_\_ DAY OF \_\_\_\_\_, 2003

BOARD OF ENVIRONMENTAL PROTECTION

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Richard Wardwell, Chair

MAINE DEPARTMENT OF ENVIRONMENTAL PROTECTION  
GENERAL PERMIT FOR ~~FINFISH~~ATLANTIC SALMON AQUACULTURE

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~~PROPOSED DRAFT~~**PART I. GENERAL CONDITONS**

- A. Authority.** A permit for the operation of a ~~n finfish~~Atlantic salmon aquaculture facility is required pursuant to Maine law, Title 38 MRSA section 413(10) and the Department's rules, Chapter 521(7). General Permits may be issued authorizing the discharge of certain pollutants pursuant to the Department's rules, Chapter 529. The Department may issue a single, general permit to a category of point sources located within the same geographic area whose discharges warrant similar pollution control measures. The similarity of discharges from ~~finfish~~Atlantic salmon aquaculture facilities has prompted the Department to issue this general permit for those waters where the assimilative capacity for the pollutants involved is relatively large in comparison to the anticipated discharge quantities. Violations of a condition of a general permit constitute a violation of the State's water quality laws and the Federal Clean Water Act, and subjects the discharger to penalties under 38 M.R.S.A. § ~~section~~ 349 and §-~~section~~ 309 of the Clean Water Act.
- B. Specialized Definitions.** In addition to the definitions found in Chapter 520 of the Department's rules, the following terms shall have the following meanings when used in this permit.
1. *Department.* "Department" means the State of Maine Department of Environmental Protection.
  2. ~~Finfish~~Atlantic salmon aquaculture facility (or "facility"). "~~Finfish~~Atlantic salmon aquaculture facility" or "facility" means a single net pen or group of net pens and appurtenances within a single leasehold granted by the Department of Marine Resources and operated by a single owner with a common management plan for the purpose of rearing Atlantic Salmon (*Salmo salar*), ~~rainbow trout (*Oncorhynchus mykiss*), Atlantic halibut (*Hippoglossus hippoglossus*), haddock (*Melanogrammus aeglefinus*), Atlantic cod (*Gadus morhua*), or other salmonid species indigenous to Maine waters.~~
  3. *Net pen.* "Net pen" means a floating structure within a leasehold granted by the Department of Marine Resources in the marine waters of the State constructed of netting, mesh or similar materials for the purpose of holding and containing ~~finfish~~Atlantic salmon.
  4. *New facility.* "New facility" means any facility commencing operation after the date of issuance of this General Permit within a leasehold that has not been used for ~~finfish~~Atlantic salmon aquaculture in the preceding five years.
  5. *Notice of Intent ("NOI").* "Notice of Intent" or "NOI" means a notification of intent to seek coverage under this General Permit made by the owner of a ~~n finfish~~Atlantic salmon aquaculture facility to the Department on a form provided by the Department.

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**C. Applicability.** Coverage under this permit is limited to those finfishAtlantic salmon aquaculture facilities that conform with the coverage requirements described below, and that have had a NOI accepted by the Department. Applicability of this General Permit is limited to activities described in the NOI that are in conformance with the terms of this General Permit. This General Permit covers only those facilities that fulfill all of the following criteria.

1. Area of coverage. This General Permit covers finfishAtlantic salmon aquaculture facilities operated in the marine waters of the State classified as SB or SC that are in compliance with the standards of those classifications and are located in the following areas:

All waters located East of Naskeag Point in Brooklin, except those waters located in the area North of a line from Schoodic Point in Winter Harbor West to Baker Island in Cranberry Isles, then West to Naskeag Point in Brooklin.

~~Fish density of facilities covered. The rearing density of a facility in kilograms of fish per cubic meter of net pen volume shall be provided in the facility's application materials and maintained at or below that level during coverage by this General Permit. The rearing density shall be low enough to avoid degradation to water quality and benthic conditions described in State water quality standards and limits contained in this General Permit. Also, the rearing density shall reflect good preventive husbandry practices designed to avoid chronic conditions in the net pens that are likely to stress cultured fish so as to elevate the risk from exposure to infectious diseases.~~

2. Effect on waters. The facility will not have a significant adverse effect on water quality or violate the standards of the receiving water's classification.
3. Current velocity. Each facility covered by this General Permit must be located in an area that has an average current velocity, as measured over at least one tidal cycle under representative oceanographic conditions, of not less than 5 cm per second at a point one half of the distance between the bottom of the net pens and the sea floor, ~~except during those periods 30 minutes before and after slack tide~~. Additionally, ~~T~~he current velocity shall, in consideration of the physical conditions at individual locations covered by this General Permit, be sufficient to avoid degradation of water quality and benthic conditions described in State water quality standards and limits contained in this General Permit.
4. Other permits required. To ~~be covered by~~operate under this General Permit, a facility must demonstrate that it has a valid, current or conditional leasehold from the Maine Department of Marine Resources pursuant to 12 MRSA section 6072, and a valid permit issued by the US Army Corps of Engineers pursuant to section 10 of the Rivers and Harbors Act of 1899.
5. Participation in monitoring programs. Each facility covered by this General Permit must participate in the Finfish Aquaculture Monitoring Program (FAMP) administered by the Department of Marine Resources.

6. Fish density of facilities covered. The rearing density of a facility in kilograms of fish per cubic meter of net pen volume shall be provided in the facility's application materials and maintained at or below that level during coverage by this General Permit. The rearing density shall be low enough to avoid degradation to water quality and benthic conditions described in State water quality standards and limits contained in this General Permit. Also, the rearing density shall reflect good preventive husbandry practices designed to avoid chronic conditions in the net pens that are likely to stress cultured fish so as to elevate the risk from exposure to infectious diseases.
7. Stratification of the water column. Facilities covered by this General Permit shall not be located in waters that demonstrate significant, persistent vertical stratification during summer months. In determining if the water column is stratified, the Department will consider testing conducted to measure temperature and density changes vertically in the water column.

#### **D. Notifications and Acceptance.**

1. Notice of Intent required. ~~Finfish~~Atlantic salmon aquaculture facilities meeting the requirements of and desiring coverage under this General Permit must submit a complete NOI with the appropriate initial permit fee to the Department. NOI forms may be obtained from the Department by contacting:

Permitting Section  
Division of Water Resource Regulation  
Bureau of Land and Water Quality  
Department of Environmental Protection  
17 State House Station  
Augusta, Maine 04333-0017  
Telephone: (207) 287-3901

Alternately, persons may obtain permitting information and forms through the internet at [www.state.me.us/dep/blwq](http://www.state.me.us/dep/blwq)

2. New facilities. Any person intending to seek coverage under this General Permit for a new facility shall notify the Department at the time an application for a leasehold is made to the Department of Marine Resources, or at least 90 days prior to the planned operation of the facility, whichever is sooner. The notice to the Department shall include information necessary for the Department to evaluate the expected impact of the new facility on existing water quality. At a minimum, this shall consist of the information referred to in sections I.D.3.c-g and II.E.4; and proposed maximum feeding rates and amounts. The Department may require additional site-specific information as necessary. Pursuant to 38 MRSA, section 464(4)(F), the Department will determine if the proposed facility may cause a significant lowering of existing water quality. In the event such a determination is made, the facility may not be covered under this General Permit and

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must apply for an individual permit from the Department. New facilities originally permitted with an individual permit must operate for a minimum of two years (i.e. following the harvest of a year class) under that permit to demonstrate that it does not have ~~an unacceptable impact~~ significant adverse effects on existing water quality before being eligible to file an NOI for coverage under this General Permit.

3. A complete NOI shall contain the following information for each individual facility.
  - a. The legal name address and telephone number of the owner of the facility;
  - b. The legal name address and telephone number of the operator of the facility if different than the owner;
  - c. The name and location of the facility, including the town and map coordinates;
  - d. A chart showing the exact location, mean low water depth and configuration of pen moorings systems and support platforms and directions of prevailing currents;
  - e. A description of the number, type, size and configuration of net pens that may be used, along with associated structures, and the minimum clearance to the sea floor.
  - f. The ~~species of fish to be reared, the~~ maximum number, stocking density and total weight of fish to be contained in the facility at any time;
  - g. A list of all drugs or medications that the facility anticipates may be used and duration, route of administration and concentration of each application;
  - h. A list of disinfectants, biocides, anti-fouling agents or other similar chemicals that may be used;
  - i. The amount, rate of use and composition of fish feed, including trace ingredients ~~ingredients~~;
  - ~~i.~~ j. A description of the system(s) to be used to dispense and monitor the consumption of feed and to detect the loss of uneaten feed;
  - ~~j.~~ k. A diagram showing intended sampling locations with GPS coordinates to meet testing requirements of this General Permit, including reference sites;
  - ~~k.~~ l. Evidence of the facility's leasehold ~~from application to~~ the Department of Marine Resources;
  - ~~l.~~ m. Evidence of the facility's permit from the Corps of Engineers;
  - ~~m.~~ n. For new facilities or relocated mooring systems where existing information is not adequate to characterize the new location, baseline monitoring data. See II.E.4 for baseline monitoring requirements;
  - ~~n.~~ o. A statement that a current Spill Prevention Control and Countermeasure plan as required by special condition II.M is available. In the case of new facilities, the plan shall be made available prior to operation of the facility;
  - p. Evidence that a public notice was published; and
  - ~~o.~~ q. The signature of an authorized person in accordance with Chapter 521 of the Department's rules.
4. Filing of a NOI. A copy of the NOI must be filed with the municipal office in which the facility is located at the time it is submitted to the Department. In the case of unorganized territories, the NOI must be sent to the appropriate County Commissioners. Additionally, a public notice shall be made in a newspaper having general circulation in the area of the project and using a format provided by the Department. This publication

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shall occur not less than 20 days prior to filing of the NOI. The Department will take no final action on the NOI until at least 30 days after the date of publication.

5. Review of a NOI and other information. Upon review of a NOI for determination of coverage under this General Permit, the Department may, at its discretion, require an applicant to apply for an individual permit. In making such a determination, the Department may consider factors, including, but not limited to, the location of the proposed facility and water quality issues particular to that area; or comments from the Department of Marine Resources, municipal officials, or the public. The Department will consider, among other things, the administrative record created by the Department of Marine Resources in granting the leasehold for the facility in determining if a facility can comply with this General Permit. The Department shall also consider any relevant information from monitoring conducted pursuant to the Department of Marine Resource's Finfish Aquaculture Monitoring Program.
6. Effective date of coverage. The Department must notify an applicant of coverage under a General Permit within 14 days of receipt of each complete NOI as to whether or not coverage for the specific discharge is permitted. If the Department does not notify the applicant within 14 days, the NOI is deemed to be accepted and coverage is granted. In the event coverage is not granted, the Department shall notify the applicant of the reasons for not granting coverage. Denial of a general permit NOI cannot be appealed. Discharges not acceptable for General Permit coverage may apply for issuance of an individual Maine Pollutant Discharge Elimination System (MEPDES) permit.
7. Transfer of ownership. In the event that the ownership of a facility is transferred to a new owner, coverage under this General Permit may be transferred by the new owner notifying the Department in writing, provided the new owner proposes no significant changes in the facility or its operation. The notice must include documentation that the new owner has title, right or interest in the facility, and the technical and financial capacity to comply with this General Permit. Such notification must be made within two weeks of the transfer. If increases or significant changes in the discharge are proposed, a new NOI must be filed.
8. Changed conditions. In the event a facility covered by this General Permit proposes to make significant changes in the nature or scope of the operations of facilities described in a NOI previously submitted, the permit holder shall notify the Department as soon as becoming aware of and before implementing such changes. Based on its evaluation of the proposed changes, the Department may require the submittal of a new NOI or that an individual permit be obtained. Significant changes include, but are not limited to, any amendment or modification to the facility's leasehold from the Department of Marine Resources that would bear on compliance with this General Permit. Reportable changes may include, but are not limited to, relocated or new mooring systems, ~~different species of fish~~ or more fish or density than indicated in the NOI.



**E. Continuing Coverage and Termination**

1. Notices by applicant and payment of fees. The term of this General Permit is for 5 years. Coverage under this General Permit will be continued upon payment of an applicable annual fee, provided there are no changes in the facility or its operation as described in the NOI. If changes occur or are proposed, the person having filed the NOI must notify the Department, as specified in I.D.6. Upon reissuance of this General Permit, persons wishing to continue coverage must so notify the Department.
2. Individual permit coverage. The Department may require, or an interested party may request, that a facility covered under this General Permit obtain an individual permit for the following reasons:
  - a. The facility is not in compliance with the conditions of this General Permit;
  - b. A change has occurred in the availability of demonstrated technology or practices for the control or abatement of pollutants applicable to the facility;
  - c. Effluent limitation guidelines are promulgated for discharges covered by this General Permit;
  - d. A Water Quality Management plan containing requirements applicable to covered facilities is approved;
  - e. Circumstances have changed since the time of the request to be covered so that the facility is no longer appropriately controlled under this General Permit, or either a temporary or permanent reduction or elimination of the authorized discharge is necessary;
  - f. A leasehold issued by the Maine Department of Marine Resources or a permit issued by the US Army Corps of Engineers is terminated, revoked or modified in such a manner so as to make compliance with the terms and intent of this General Permit impractical;
  - g. The Department determines that additional monitoring beyond that required in this General Permit is necessary to properly characterize impacts of the facility's operation;
  - h. The facility is a significant contributor of pollutants. In making this determination, the Department may consider the following factors:
    - (1) The location of the facility with respect to waters of the State;
    - (2) The size of the discharge; and
    - (3) The quantity and nature of the pollutants discharged to waters of the State; or
  - i. Any other factors that the Department deems relevant.
3. A facility may request that it be excluded from coverage under this General Permit and apply for an individual permit pursuant to Chapter 529 (3)(iii-v) of the Department's rules. When an individual MEPDES permit is issued to an operator otherwise subject to this general permit, the applicability of this permit to that owner or operator is automatically terminated on the effective date of the individual permit.

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FinfishAtlantic salmon aquaculture facilities obtaining coverage under this General Permit are subject to the discharge limitations, monitoring requirements and management practices specified in the following sections.

- A. General limitations.** A facility may discharge from discrete floating net pens the following: fish excrement, fish feed, and drugs ~~approved by the Food and Drug Administration (FDA) for finfish aquacultural purposes that are applied in accordance with federal and state laws~~pursuant to section II.L, Use of Drugs for Disease Control. Additionally, other discharges incidental to the normal and proper operation of the facility, such as the loss of fish scales and treatment compounds used on structures and vessels to limit marine growth, may occur provided they do not ~~significantly affect~~have significant adverse effects on water quality. ~~See II.L, Best Management Practices for Disease Control.~~

Domestic waste shall not be discharged and must be collected and transported to a land-based facility authorized to dispose domestic waste.

- B. Feeding rates and monitoring.** The discharge of fish feed shall be at the minimum amount necessary to sustain an optimal rate of fish growth with the minimum loss of uneaten feed, ~~but in no circumstance shall feeding exceed a total of 1.3 kg of feed for each kg~~For each year class, the facility shall report its Food Conversion Ratio (FCR) as kg of feed used per kg of live weight of fish harvested or lost over the time those fish are confined to net pens. In calculating the FCR, the facility may use processing facility and gut loss information provided that records supporting the FCR determination are made available to the Department for review and approval. Fish maintained in separate net pens as broodstock need not be included in FCR calculations. Each facility shall maintain a real-time monitoring system designed to track the rate of feed consumption and detect uneaten feed passing through the net pens. Such systems include, but are not limited to, doppler radar detection or video cameras. There shall not be any significant accumulation of unconsumed feed on the sea floor beneath or adjacent to net pens.

- C. Mixing zones.** This permit designates two mixing zones: (1) a Water Column Mixing Zone, and (2) a Sediment Mixing Zone. Outside the allocated Mixing Zones, discharges from the facility shall not cause or contribute to conditions that are hazardous or toxic to aquatic life, or would impair the uses designated by the classification of the receiving waters. At individual facilities, the location of the mixing zones may be shifted to reflect the effect of currents unique to a specific site, provided that the offset mixing zones are no larger in area than those defined by the size of the net pen(s).

1. **Water Column Mixing Zone.** The Water Column Mixing Zone is defined as the area within and extending 30 meters beyond the perimeter of a net pen in all directions on the surface, and down to the sea floor/water column interface.

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2. Sediment Mixing Zone. The Sediment Mixing Zone is defined as the sea floor directly below a net pen and extending on the sea floor 30 meters beyond the perimeter of each net pen in all directions. See II.G for limitations on changes that may occur within ~~segments of~~ the Sediment Mixing Zone.

**D. Narrative limitations.** Operation of a facility under authority of this General Permit shall at all times comply with the State's water quality laws, including, but not limited to the following narrative limitations that apply to waters beyond the designated Water Column Mixing and Sediment Mixing Zones.

1. The discharges shall not cause a visible oil sheen, foam, or floating solids at any time that would impair the uses designated by the classification of the receiving waters;
2. Discharges from the facility shall not contain materials in concentrations or combinations that are hazardous or toxic to aquatic life, or that would impair the existing or designated uses of the receiving waters;
3. The discharges shall not cause toxicity, visible discoloration, turbidity or other effects to the receiving water that would impair the existing or designated uses of the receiving waters;
4. The facility shall not discharge suspended or settleable solids that will ~~adversely affect~~ have significant adverse effects on the quality resources, or ~~impair~~ any uses of the receiving water body;
5. The discharges shall not produce or result in harmful algae blooms that may be characterized by excessive growths of, but not limited to, the genera *Alexandrium*, *Dinophysis*, *Prorocentrum*, *Pseudonitzschia*, *Phaeocystis*, *Enteromorpha*, *Ulva* or *Aureococcus*; and
6. Notwithstanding compliance with specific conditions of this General Permit, the discharge shall not cause or contribute to violations of water quality standards.

**E. Monitoring Requirements**

1. Levels of monitoring and general requirements. Each facility covered by this General Permit shall conduct periodic monitoring for ambient water quality, benthic analysis, biological assessment and video/photo surveys at the frequency determined by its respective monitoring level. Based on the results of monitoring or site--specific conditions, the Department may require a facility to conduct additional monitoring to determine compliance with this General Permit and applicable statutory requirements.
  - a. Monitoring levels. The type and frequency of monitoring is defined by the following monitoring levels.

Level I	Those facilities located in the waters of Cobscook Bay, North or inland of West Quoddy Head in Lubec.
Level II	Those facilities located in waters between West Quoddy Head in the Lubec and Naskeag Point in Brooklin that are covered by this General Permit.

- b. Summary of limitations and monitoring requirements. The following table is a summary of the monitoring required by the various Special Conditions below.

<u>Special Condition</u>	<u>Topic</u>
II.E.5	Fish feeding and monitoring
II.E.6	Near-field water quality monitoring requirements
II.E.7	Far-field and reference site monitoring requirements
II.E.8	Video and photographic monitoring requirements
II.E.9	Sediment and benthic monitoring requirements

2. Sampling information. For all water column and benthic monitoring samples collected, the facility shall measure and maintain records of the following information. All monitoring information and records required by the General Permit shall be kept current at all times and made available to representatives of the Department or the Department of Marine Resources upon request.
  - a. The sampling location, recorded as latitude and longitude to the nearest one-tenth second.
  - b. The date and time of day.
  - c. The current direction in ~~degrees magnetic~~ relation to true north.
  - d. The tidal stage to the nearest one-half meter above/below mean low water.
  - e. The depth of water.
3. Modification of monitoring requirements. The Department may, after public notice and opportunity for hearing, modify this General Permit to assure that sufficient information is available to determine compliance with applicable water quality standards and the terms and conditions of this General Permit. Modifications may also be made to assure monitoring required by the General Permit is, to the maximum extent possible, consistent with the Finfish Aquaculture Monitoring Program administered by the Maine Department of Marine Resources.
4. Baseline monitoring. Before a facility commences operation at a site not used for ~~finfish~~ aquaculture in the previous 5 years, the facility shall submit baseline site information describing the intended site to the Department. This information shall include the site's location, water depth, temperature, salinity, current flow, dissolved oxygen profiles, bottom type(s), sediment grain size, evidence of out-gassing, sediment Redox potential, sulfide, copper, zinc and a description of the kinds and abundance of flora and fauna present to the lowest practical taxonomic level at sufficient locations to fully describe conditions at the site. A minimum of three benthic baseline samples shall be collected for each bottom type at the facility location and analyzed to allow for statistical comparisons. Baseline sampling shall be conducted in accordance with a sampling plan approved by the Department, and shall be done during the months of July through September, unless otherwise approved by the Department. To the extent relevant and acceptable data are available from previous studies, they may be used.

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5. Feed discharge and fish monitoring requirements. The facility shall maintain and report monthly to the Department the following information.
  - a. The number of net pens in use, including type, size and configuration;
  - b. The age, weight and number of fish in each net pen;
  - c. The number and total weight of fish contained in all net pens in use;
  - d. The total amount of feed added to each net pen; and
  - e. The total amount of feed added to all net pens.

6. **Near-field water quality monitoring requirements.** During the period of **June 1 through September 30-October 31** each year, the facility shall maintain the specified conditions and conduct the following monitoring of the ambient water **within the water column mixing zone** for each contiguous group of net pens. All samples shall be grab samples at locations selected to represent the greatest level of any impact of the facility's operation.

Table E.6. Monitoring Requirements at locations within 30-5 meters down-current of the pens (prevailing conditions at the time of sampling).

Sea Water Characteristic	Location			Frequency	
	Mid-Net Pen Depth	Mid-Water Column Depth	One Meter Above Sea Floor	Level I	Level II
Dissolved Oxygen Concentration	$\geq 6$ mg/L	$\geq 6$ mg/L	$\geq 6$ mg/L	<del>1</del> 2/month	<del>2/month</del> 1/week
Dissolved Oxygen Saturation	Report %	Report %	Report %	<del>1</del> 2/month	<del>1/week</del> 2/month
Salinity	Report ‰	Report ‰	Report ‰	<del>1</del> 2/month	<del>1/week</del> 2/month
Temperature	Report ° C	Report ° C	Report ° C	<del>1</del> 2/month	<del>1/week</del> 2/month
Transparency	Report meters			<del>1</del> 2/month	<del>1/week</del> 2/month

- a. **Sampling Locations.** Samples collected in compliance with the above monitoring requirements shall be taken within ~~the water column mixing zone~~ 5 meters of the net pens and down-current (prevailing conditions at the time of sampling), from where water passes through pens stocked with fish and at a point selected to best represent the maximum impact of the facility's operation. The samples shall be taken at mid-pen depth (i.e. if the containment net is 6 meters deep, take the sample at 3 meters from the surface), mid-water column depth, and within 1 meter of the sea floor, where each station's results are reported separately.

- b. Sampling methods. Water samples shall be analyzed for dissolved oxygen (DO) concentration and saturation, temperature and salinity at the frequencies specified above. Measurements of temperature and salinity in part per thousand shall be used to determine percent saturation of dissolved oxygen and stratification. Samples should be taken within one hour before or after slack water prior to 9:00 AM in the morning. If the frequency of sampling is increased such that early morning slack tide measurements cannot be made, samples shall be collected prior to 9:00 AM, irrespective of tidal conditions. All measurements shall be made using approved methods, and in accordance with the applicable manufacturer's instructions, including calibration of instruments. The depths of all measurements shall be recorded to at least the nearest one-half meter.
- c. Transparency readings shall be made by lowering a 30 cm Secchi disk vertically through the water column. Observations are to be made using a viewing scope to penetrate the surface of the water. The depth of disappearance upon descent and reappearance upon retrieval of the disk shall be measured and averaged.
- d. Compliance with DO concentration. If DO concentrations below 6 mg/L are recorded at any depth, additional samples shall be taken to determine if the DO depression is a result of the facility's discharge. To determine if DO depression below 6 mg/L reflects natural conditions, readings must be taken at the far--field and reference sites (see II.E.7 and II.F) at the comparable times, depths and tidal conditions and reported to the Department with Discharge Monitoring Reports. Further, at any time the DO saturation is less than 85% in Class SB waters or less than 70% in Class SC waters as measured within 5 meters of the net pens, far--field monitoring described in II.E.7 shall be conducted.
- e. Reduced monitoring. After at least two years of monitoring, including one year when market size fish are on site, the Department may reduce monitoring for individual facilities to 1/month for Level I and 2/month for Level II, provided that all results demonstrate compliance with percent saturation standards for the respective class of water. In considering reduced monitoring, the Department will evaluate factors that may influence the representativeness of monitoring reports, such as the total number of fish on site, stocking density, water temperature or unusual environmental conditions.

7. **Far-field and reference site water quality monitoring requirements.** During the period of **June 1 through September 30/October 31** each year, the facility shall maintain the specified conditions and conduct the following monitoring of the ambient water adjoining each contiguous group of net pens. All samples shall be ~~grab samples~~vertical profiles measured at intervals of 1 meter or less at ~~a~~ locations selected to represent the greatest level of any impact of the facility's operation. Additional monitoring may be required pursuant to section II.E.6.d.

Table E.7. Far-field and reference site water quality monitoring requirements.

Sea Water Characteristic	<del>Monitoring at each site—Depth Below Water Surface</del>			Sampling Frequency	
	<u>Minimum value</u> <del>Mid-Net Pen</del> <u>Depth</u>	<u>Average value</u> <del>Mid-Water</del> <u>Column-Depth</u>	<u>Maximum value</u> <del>One-Meter</del> <u>Above Sea Floor</u>	Level I	Level II
Dissolved Oxygen Concentration	Report mg/l	Report mg/l	Report mg/l	1/ year in August	2/ year in <del>July and-August</del> <u>and September</u>
Dissolved Oxygen Saturation	Class SB: 85% Class SC: 70%	<del>Class SB: 85%</del> <u>Class SC: 70%</u> <u>Report %</u>	<del>Class SB: 85%</del> <u>Class SC: 70%</u> <u>Report %</u>	1/ year in August	2/ year in <del>July and-August</del> <u>and September</u>
Temperature	Report ° C	Report ° C	Report ° C	1/ year in August	2/ year in <del>July and-August</del> <u>and September</u>
Salinity	Report ‰	Report ‰	Report ‰	1/ year in August	2/ year in <del>July and-August</del> <u>and September</u>
Transparency	Report meters			1/ year in August	2/ year in <del>July and-August</del> <u>and September</u>

- a. Sampling locations. Samples collected in compliance with the above monitoring requirements shall be taken at a position ~~100 meters or less~~approximately 30 meters down-current (prevailing conditions at the time of sampling); from where



water passes through pens stocked with fish and at a point selected to best represent the maximum impact of the facility's operation. As required by the Department, samples shall also be collected at the reference site (see II.F). ~~The samples shall be taken at mid pen depth (i.e. if the containment net is 6 meters deep, take the sample at 3 meters from the surface), mid water column depth, and within 1 meter of the seafloor.~~ All information recorded in profiles shall be reported to the Department.

- b. Sampling methods. Water samples shall be analyzed for dissolved oxygen (DO) concentration and saturation, temperature and salinity at the frequencies specified above. Measurements of temperature and salinity in part per thousand shall be used to determine percent saturation of dissolved oxygen. Samples should be taken one hour before or after slack water prior to 9:00 AM in the morning. If the frequency sampling is increased such that early morning slack tide measurements cannot be made, samples shall be collected prior to 9:00 AM, irrespective of tidal conditions. All measurements shall be made using approved methods, and in accordance with the applicable manufacture's instructions, including calibration of instruments. The depths of all measurements shall be recorded to at least the nearest one-half meter.
  - c. If DO saturation levels below 85% in Class SB waters or 70% in Class SC waters are recorded at any depth, additional samples shall be taken to determine if the DO depression is a result of the facility's discharge. In order to determine if DO depression below the specified saturation reflects natural conditions, readings must be taken at the reference site (see II.F) at the comparable times, depths and tidal conditions and reported to the Department with Discharge Monitoring Reports.
  - d. Transparency readings shall be made by lowering a 30 cm Secchi disk vertically through the water column. Observations are to be made using a viewing scope to penetrate the surface of the water. The depth of disappearance upon descent and reappearance upon retrieval of the disk shall be measured and averaged.
8. **Video and photographic monitoring requirements.** Twice per year: once in April or May and again in August through October, the facility shall conduct color video or photographic evaluation of the sea floor under and adjacent to each net pen system as follows. Multiple evaluations may be needed where independent pens or systems ~~are in use~~ preclude coverage by one transect. The Department may waive the spring monitoring for individual facilities when monitoring the preceding fall indicates that the warning levels specified in II.G are not exceeded and there are no other indications of impact from the facility's operation. Monitoring and evaluation shall be conducted in accordance with methods in the Department of Marine Resource's Finfish Aquaculture Monitoring Program, unless otherwise specified herein.

Table E.8. Video and photographic monitoring requirements.

Monitoring Characteristic	Substrate Video Monitoring Transects
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	Transect Beneath Pens	Transect 60 m Up-current From Edge of Pens	Transect 60 m Down-current From Edge of Pens	Frequency
Video Tapes and/or Photographs of Substrate	Report	Report	Report	2 per year (all Levels)

- a. Reports of monitoring shall include the date(s) on which monitoring was conducted and the video tapes or photographs, along with all supporting information including a site schematic of the video track or still photo locations in relation to the net pens. The beginning and ending points of transects shall be located by GPS. Reports shall be submitted to the Department within 90 days of the monitoring event.
- b. Except as provided below, the survey shall be documented with continuous video footage. The filmed survey shall document the sediment type and color, as well as features, noting erosional or depositional areas. The survey shall also document the flora/fauna observed as to their relative abundance, as well as any feed pellets or other man-made debris. The presence of *Beggiatoa* type mats shall be noted, and its growth described as light, moderate, or heavy. - Black sediments, spontaneous or induced gassing, or the presence of pimpled sediments shall be noted. "Pimpled" sediments may also represent the presence of infauna, and as such, will not be used exclusively as an Impact Limit unless such marks are readily distinguishable from infauna burrows. The location of any N-nets located on the bottom shall be positioned documented relative to the pen system, and the extent to which the net is buried beneath sediments shall be noted. Relative abundance of *Beggiatoa* should be characterized approximately as follows: abundant, frequently present within the film coverage; common, seen occasionally throughout the film coverage or existing in patches; rare, only seen once or in a few places throughout the dive.
- c. If water depths at a facility exceed the State of Maine's safe working depth limit of 85 feet for SCUBA diving, video surveys normally conducted by divers may instead be obtained using one or more of the following methods: a video camera mounted on a tethered sled, a tethered drop still camera, tethered drop video camera or equivalent. -If still photos are taken with a tethered camera, one photograph shall be taken at least every 10 meters along each transect.
- d. A video/photo transect shall be conducted beneath the pens (or, if not possible due to depths beyond 85 feet or physical constraints, directly adjacent to the up-current edge of the pens) along an axis representing the direction of the prevailing current, and extend 60 meters beyond the pen system on each end, and located to best reflect the extent of the facility's

impact on benthic conditions. Video coverage of sediments beneath or adjacent to feed or service barges shall be noted on the film narrative.

- e. The film coverage shall be in color, and of sufficient detail and clarity to allow for the accurate assessment of benthic conditions. The camera should be positioned at a height above the substrate that will provide approximately one square meter of bottom coverage, and be illuminated with sufficient artificial light to enable the accurate identification of epibenthic organisms and sediment conditions. A brief written narrative with the tape or photos describing reference points shall be provided. All film documentation shall include the dates on which it was taken, the direction of the current, and the geographic positions of the start and endpoints of the transects.

9. **Sediment and benthic monitoring requirements.** The facility shall conduct monitoring of the sediments on the sea floor as follows. ~~All samples shall be sediment grab cores collected during August through October of years in which fish are contained in the net pens are near the maximum biomass during the growing cycle.~~ Benthic monitoring shall focus on sediment conditions and the infaunal community. The reference site is described in II.F. The Department may require that the monitoring required by this condition be continued following removal or relocation of a net pen as necessary to evaluate residual impacts. Monitoring and evaluation shall be conducted in accordance with the Department of Marine Resource's Finfish Aquaculture Monitoring Program, unless otherwise specified herein.

Table E.9. Sediment and benthic monitoring requirements.

Monitoring Characteristic	Sample Location				Monitoring Frequency All Levels
	Within the <del>net</del> pen system mixing zone	<del>Within</del> 30 m of <del>from</del> net pens	<del>Within 60 m</del> of <del>net pens</del>	Reference Site*	
Redox Potential	Report mV	Report mV	<del>Report mV</del>	Report mV	<u>2/year in Apr-May and Aug-Oct</u> <del>Once per 2 years</del>
<u>Sulfide</u>	<u>Report uM</u>	<u>Report uM</u>		<u>Report uM</u>	<u>2/year in Apr-May and Aug-Oct</u>

Anoxic Sediments, Gas Formation, and <i>Beggiatoa</i>	Report	Report	<u>Report</u>	Report	<u>2/year in Apr-May and Aug-Oct</u> <u>Once per 2 years</u>
Azoic Conditions	Report /0.1 square m	Report /0.1 square m	<u>Report</u> <del>/0.1 square m</del>	Report /0.1 square m	<u>1/5 years and as required, see II.E.9.a</u> <u>Once per 2 years</u>
Taxa Present, Absolute, <del>and</del> Relative Abundance, <del>and</del> <u>Shannon-Wiener Diversity Index</u>	Report /0.1 square m	Report /0.1 square m	<u>Report</u> <del>/0.1 square m</del>	Report /0.1 square m	<u>1/5 years and as required, see II.E.9.a</u> <u>Once per 2 years</u>
<u>Sediment grain size</u>	<u>Report % sand, silt, clay or gravel</u>	<u>Report % sand, silt, clay or gravel</u>		<u>Report % sand, silt, clay or gravel</u>	<u>When taxa measurements are made</u>
<u>Total Organic Carbon in Sediment</u>	<u>Report, mg/g</u>	<u>Report, mg/g</u>		<u>Report, mg/g</u>	<u>When taxa measurements are made</u>
Copper, Total metal	Report mg/kg Dry weight	Report mg/kg Dry weight	<del>Report mg/kg Dry weight</del>	Report mg/kg Dry weight	Once per 2 years
Zinc, Total metal	Report mg/kg Dry weight	Report mg/kg Dry weight	<del>Report mg/kg Dry weight</del>	Report mg/kg Dry weight	Once per 2 years
Medications used	Report ug/kg Dry weight	Report ug/kg Dry weight	<del>Report ug/kg Dry weight</del>	Report ug/kg Dry weight	Within <u>six-one</u> months of use <del>(all Levels)</del>

- a. Sampling locations and times. ~~Sediment and infauna samples shall be collected at the same locations. Samples shall be collected beneath the each net pen system and within 30 and 60 meters on both sides of the net pens along an axis representing the direction of the prevailing flow of the current. The number and location of the samples shall be determined by pen configuration, visual inspection, husbandry practice including the use of drugs, and previous monitoring history in order to best reflect the extent of the facility's impact on benthic conditions. Samples sites will be selected to include the greatest level of any impact of the facility's operation. The location (i.e., GPS coordinates and reference to the pen system) shall be recorded at each sample site. Samples for all parameters shall be taken at the same locations. Sampling locations shall be along a transect as described for video monitoring, II.E.8.d, above. There shall be a minimum~~

of 4 locations along the transect, 2 on each side of the net pens. On each side of the net pen system, one location shall be taken at 30 meters from the pens and will represent conditions outside of the mixing zone. Another sample shall be taken within the mixing zone where benthic impact is observed to be the greatest; if no differences in impact can be observed, the location shall be approximately 5 meters from the pens. At each location, a minimum of 3 individual samples shall be collected along a line perpendicular to the transect line, and spaced at distances reflecting and within the lateral extent of the greatest benthic impacts at that location. In order to fully evaluate conditions, the Department may require additional sampling locations on a case--by case--basis.

Benthic sampling shall be done at the same time as the video observations are made. See II.E.9.f for testing of medications. At a minimum, azoic conditions and taxa measurements shall be conducted in August – October once during a 5 year period in a year when fish in the facility are near their maximum biomass. Additionally, these measurements shall be made at any time a warning level in section II.G is exceeded, unless the Department determines that the cause of the exceedence has been corrected pursuant to II.G. Copper and zinc are to be tested once per two years in a year when fish in the facility are near their maximum biomass, and reports shall include the percent solids of the sediment sampled. Other measurements shall be conducted twice per year, in April – May and August – October. However, the Department may waive the April – May benthic sampling if the video monitoring is waived pursuant to section II.E.8.

\* See Section II.F for when reference sites must be sampled.

- b. Reports shall include the date(s) of the sampling and the results of the analyses, along with all supporting information including a site schematic of the sample locations. Reports shall be submitted to the Department within ~~270~~150 days of the monitoring event. However, based on prior benthic monitoring, video monitoring, or other information that indicate the facility may be adversely impacting the sediment, the Department may require earlier submission of benthic monitoring reports.
- c. Each grab sample shall be inspected for evidence of anoxia, the presence of *Beggiatoa* type bacterial mats, and gas formation (hydrogen sulfide or methane). The surface color of the sediment sample (specifically, sediments are black or significantly darker than natural sediment in the area), and any evidence of gas formation (e.g. pimpled sediments, hydrogen sulfide odor) or *Beggiatoa* shall be reported. If subsamples are taken from a grab or box type corer for the sediment analysis and the remaining sample used for infauna analysis, no more than one-quarter of the surface of each sample can have been removed for the sediment analysis.

- d. Cores for metals or medications must be of the top 2 cm, and in the top 3 cm for Redox potential and sulfide. Single cores 4 inches or greater in diameter shall be taken from the sediment for infauna and must be inserted to resistance or 15 cm, whichever is less. Depth of the core shall be reported. Infauna samples shall be sieved through a 1.0 mm mesh sieve. Organisms shall be fixed in 10% buffered formalin and stained with a 1% Rose Bengal staining solution. After 5 days in the formalin solution, the formalin shall be replaced with 70% ethanol to ensure preservation of the organism's integrity. Organisms shall be identified to the family or a lower practical taxonomic level and enumerated. The Department may require more specific identification of organisms in order to determine compliance with this General Permit. A conversion coefficient shall be developed to convert the core sample surface area to 0.1m<sup>2</sup> for reporting family abundance and richness.
- e. Sediment sample collection, handling, preservation, storage, and analysis shall be conducted in accordance with EPA approved methods. See references listed in Part III (EPA 1995, and 1986-1991) for appropriate guidance.
- f. Tests for medications shall be conducted for each medication used within ~~six-one~~ months of such use and shall include analysis for the compound(s) used and any known primary metabolites. The Department may waive this testing if the facility provides information demonstrating that medications used do not accumulate in the sediments or organisms.

**F. Reference sites.** The facility shall maintain reference sites and baseline information approved by the Department to provide comparative information on water quality and benthic conditions in the area of the net pens. Where sufficiently detailed and relevant baseline data are available (see section II.E.4), those data may be used with reference station data for comparative information in evaluating the results of benthic monitoring tests. If baseline benthic data are incomplete or no longer representative, and for water column information, reference site sampling shall be conducted in order to establish comparative information. The Department may require repeat or on-going reference site monitoring as necessary to properly evaluate the results of monitoring data. Each reference site will be selected to best represent local conditions free of influences from the activities of the facility or other uses of the receiving water in the vicinity of the facility. A water column reference site shall be located approximately at least 100 meters from the facility and perpendicular to an axis representing the direction of the prevailing current flow. A benthic reference site shall be similarly located and selected to have physical characteristics of the sea floor as close as possible to those used for monitoring pursuant to II.E.9. For each type of bottom, at least three benthic samples shall be collected and analyzed to allow for statistical comparisons. -The Notice of Intent filed by the facility for coverage under this General Permit shall identify the locations of reference sites and other activities within 1000 meters that could influence water quality (e.g. marinas, other aquaculture facilities, point source discharges, etc.). In consideration of such activities, facilities may utilize reference sites that are further from the net pens, but at no greater distance than is necessary to

avoid the influence of the activities. The Department may require additional reference sites to be used where necessary to adequately characterize conditions in an individual location.



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**G. Impact thresholds.** With respect to the sediment and benthic monitoring specified in II.E.8 and II.E.9, the following criteria will be applied by the Department in determining if discharges from a facility are causing or contributing to impairment of the State's water quality criteria. ~~The distances are from the edge of the net pen(s).~~

~~Table G.1. Sediment Mixing Zone impact thresholds under and within 5m of net pen(s)~~

<u>Metric</u>	<u>Warning Level</u>	<u>Impact Limit</u>
Redox Potential <sup>†</sup>	Mean value 100 to 0 mV nhe	Mean value < 100 mV nhe <sup>‡</sup>
Gas Formation	Presence of anoxic sediments <sup>‡</sup>	Compelling evidence <sup>2,‡</sup>
<i>Beggiatoa</i> Coverage	Patchy <sup>3</sup>	≥ 50% photo coverage <sup>4,‡</sup>
Anoxic Sediments	Patchy <sup>3</sup>	≥ 50% photo coverage <sup>4,‡</sup>
Pollution-Tolerant Taxa <sup>5</sup>	Number of individuals in single taxa > 90%	Report information
Azoic Conditions	> 50% reduction in mean abundance as compared to baseline or reference site values	Absence of infauna <sup>‡</sup>

**PROPOSED DRAFT**Table G.21. Sediment Mixing Zone impact thresholds ~~between 5m under or and within~~ 30\_m of net pen(s).

Metric	Warning Level	Impact Limit
Redox Potential <sup>1</sup>	Mean value -100 to <del>100-0</del> mV nhe <sup>7</sup>	Mean value <-100 mV nhe <sup>7</sup>
<del>Sulfide</del> <sup>1</sup>	<del>Mean 1300 – 6000 uM</del>	<del>Mean &gt;6000 uM</del>
<del>Gas Formation</del>	<del>Presence of anoxic sediments</del> <sup>7</sup>	<del>Compelling evidence</del> <sup>2,7</sup>
<i>Beggiatoa</i> Coverage	<del>&gt;25% photo coverage</del> <sup>4, 7</sup> <del>Patchy</del> <sup>3</sup>	≥ 50% photo coverage <sup>4, 7</sup>
Anoxic Sediments <sup>3</sup>	<del>&gt;25% photo coverage</del> <sup>4, 7</sup> <del>Patchy</del> <sup>3</sup>	≥ 50% photo coverage <sup>4, 7</sup>
Pollution-Tolerant Taxa <sup>5</sup>	Number of individuals in single taxa > 70%	Report information
	AND	
Pollution-Sensitive Taxa <sup>6</sup>	>50% reduction in mean abundance of taxa not identified as pollution-tolerant	Report information
Taxa richness	>25% reduction in total number of all taxa compared to mean baseline or reference site	Report information
Azoic conditions	>50% reduction in total abundance compared to mean baseline or reference site	Absence of infauna <sup>7</sup>

Table G.32. Sediment Impact Thresholds Beyond Sediment Mixing Zone ( $\geq 30$  m from the nets pens).

Metric	Impact Limit
Redox Potential <sup>1</sup>	Report information
<a href="#">Sulfide</a>	<a href="#">Report information</a>
<a href="#">Gas Formation</a>	<a href="#">Compelling evidence</a> <sup>2, 7</sup>
<i>Beggiatoa</i> Coverage	Compelling evidence <sup>2, 7</sup>
Anoxic Sediments	Compelling evidence <sup>2, 7</sup>
Pollution-Sensitive Taxa <sup>6</sup>	SB waters: Significant decrease in mean number of listed taxa as compared to mean baseline or reference site value <sup>8</sup> SC waters: Unsuitable for any species of indigenous fish, or the structure and function of the resident biological community is not maintained
Taxa Richness	SB waters: Significant decrease in mean number of total taxa as compared to mean baseline or reference site value <sup>8</sup> SC waters: Unsuitable for any species of indigenous fish, or the structure and function of the resident biological community is not maintained

Footnotes to Tables G.1, [and G.2](#) ~~and G.3~~:

<sup>1</sup> Redox Potentials (Eh) shall be measured in millivolts (mV) relative to the normal hydrogen electrode (nhe) for the top 3 cm of the sediment profile. See Wildish et. al. 1999 for an acceptable approach to redox sampling, analysis and instrument calibration. [Mean values for redox and sulfide shall be the average of all individual samples collected at a location a given distance from the net pens.](#)

<sup>2</sup> Compelling evidence includes photo or video documentation, diver observations, or sediment analyses that reveals actual off-gassing, or evidence of gas formation including “pimpled” sediments and the smell of hydrogen sulfide gas emitted from grab samples or the presence of *Beggiatoa*, and such conditions are not observed in the baseline or reference site, or are the result of natural conditions.

<sup>3</sup> ~~*Beggiatoa* or anoxic sediments shall be considered “patchy” if coverage is non continuous and occupies less than 50% of the area, as determined by the Department from the review of video footage and/or photographs taken beneath or adjacent to each pen, and the presence is not result of natural conditions. Anoxic sediments consist of black or significantly darkened~~

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sediment in comparison to natural conditions in the area, and/or the formation of hydrogen sulfide or methane gas as characterized by emission of gas bubbles, “pimpled sediments” or odors in the sediment.

<sup>4</sup> Percent cover shall be determined by the Department from the review of video footage and/ or photographs taken beneath or adjacent to each pen.

<sup>5</sup> Pollution-tolerant taxa include the following the Polychaetes: *Capitella capitata*, Oligochaetes and other taxa that may be present as determined from baseline information and/or the reference site.

<sup>6</sup> A list of pollution-sensitive taxa is to be determined from pre-operation baseline studies and/or the reference site specified in this permit. Such species include, but are not limited to, amphipods and cumaceans.

<sup>7</sup> Unless similar abundance or values exist in the baseline or reference site specified in this permit, or are the result of natural conditions.

<sup>8</sup> The significance will be evaluated based on statistical analysis at a confidence interval acceptable to by the Department, ~~through comparative analysis of site specific data and expected conditions for similar unimpacted sites and meeting generally accepted professional standards.~~

The forgoing impact limits represent one definition of conditions that would represent non-attainment of narrative water quality standards. To assess compliance, the Department may consider the results of monitoring conducted pursuant to this permit, the conditions found in the baseline or reference site for comparative purposes and other available information. This information may include, but is not limited to, total abundance, relative abundance, diversity indices, dominant taxa, the percentage of mollusks, echinoderms and crustaceans, and trophic levels. In doing so, the Department may determine that other conditions found at an individual location may constitute a violation of narrative water quality standards.

Physical disturbance such as harrowing, dragging, or other mechanical means shall not be used to mitigate bottom conditions.

The facility shall notify the Department as soon as it has reason to believe the warning levels that are specified for the Sediment Mixing Zone may be exceeded. At that time, or upon notification by the Department, the facility shall review its past operations and propose any changes that it deems to be necessary to assure that impact levels are not exceeded. If the degree by which warning levels are exceeded in subsequent monitoring events is increased, or if an impact level is exceeded at any time, the facility shall include in its notification, for the Department's for review and approval, a plan and implementation schedule for modification of operations. Such modifications may include, but are not limited to, reducing standing stock, reduced feeding, fallowing of the site and/or collection of settled materials before they reach the sea floor. New fish shall not be stocked into pens at the facility until the approved plan has been implemented. The Department may require additional

monitoring to determine the effectiveness of these measures or continuing trends in benthic conditions.

## H. Toxic impacts

1. The discharge of toxics into the waters of the State in concentrations identified by the Department as toxic to aquatic organisms is prohibited. When waters are temporarily contained within a barrier, such as a plastic tarpaulin, for the application of medications, at the point the barrier is removed the concentration of those medications shall not pose a risk of causing lethal effects on organisms passing through the water column. Within the water column mixing zone, acutely toxic (lethal response) conditions must not occur. At the edge of the water column mixing zone concentrations of any compound cannot exceed levels known to cause acute or chronic toxicity to marine organisms, or sub-lethal effects from repeated exposure.
2. Sediments within or beyond the Sediment Mixing Zone shall not contain toxics originating from the facility in concentrations or combinations that are likely to have ~~an~~ a significant adverse ~~impact to effect on~~ benthic infauna or epifauna, or bio-accumulate in organisms such that those organisms can ~~impact adversely~~ have a significant adverse effect on marine life that prey upon them. Such marine life includes, but is not limited to, demersal finfish, lobster, and marine mammals.

## I. Protection of Atlantic Salmon. ~~The Special Conditions in this section are applicable only to facilities that contain Atlantic salmon~~The Department may, after public notice and opportunity for hearing, modify this General Permit to consider new information regarding the protection of Atlantic salmon or relevant conditions that may be imposed by the US Army Corps of Engineers.

1. a. ~~Effective July 31, 2003, all Atlantic salmon placed in net pens must be of North American origin. Except as described in this section, the use of Atlantic salmon originating from non-North American stock is prohibited.~~ Non-North American stock is defined as any Atlantic salmon (*Salmo salar*) that possess genetic material derived partially (hybrids) or entirely (purebreds) from any Atlantic salmon stocks of non-North American heritage, regardless of the number of generations that have passed since the initial introduction of the non-North American genetic material. For the purposes of this permit, classification of brood fish as either North American or non-North American stock will be based on genetic evaluation of each fish's DNA in accordance with Appendix A, Atlantic Salmon Microsatellite Analysis Protocol. The Microsatellite Protocol shall be used to classify each brood fish and only the progeny of brood fish classified as North American stock will be allowed in net pens.

If sub-samples of a population are to be used to demonstrate compliance with Appendix A, the sub-samples shall be demonstrated to be a statistically valid representation of the population and the sampling scheme shall be approved by the National Marine Fisheries Service and/or the U.S. Fish and Wildlife Service.

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- b. Only individual fish determined to be North American, according to Appendix A, can be used to produce offspring to be placed in net pens. No fish classified as non-North American according to Appendix A can be utilized to create progeny for stocking in net pens.
- bc. Prior to ~~March~~ January 1 of each year, beginning in ~~2003~~ 2004, genetic evaluation information developed pursuant to Appendix A shall be submitted to the National Marine Fisheries Service and/or the US Fish and Wildlife Service, with confirmation sent to the Department.
- ed. Prior to ~~June 30~~ March 1 of each year, beginning in ~~2003~~ 2004, the facility shall submit to the Department a letter from the National Marine Fisheries Service and/or the US Fish and Wildlife Service certifying ~~that it will be in compliance with condition 1-a~~ the results of the genetic evaluation information submitted pursuant to II.I.1.c, above. In the event any fish or gametes are found to be non-North American pursuant to Appendix A, the facility shall also report to the Department the disposition of those fish or gametes.
- e. Effective July 31, 2004, all Atlantic salmon placed in net pens must be of North American origin. Within 30 days placement of fish, the facility shall provide the Department with written confirmation regarding compliance with this condition.
- f. ~~d.~~ All non-North American Atlantic salmon must be removed from net pens prior to September 15, 2006. Within 30 days of removal of fish, the facility shall provide the Department with written confirmation regarding compliance with this condition.
- g. Prior to November 1, 2003, if an existing facility is unable to comply with II.I.1.b, e or f, above, it may submit to the Department for review and approval a request for an alternate compliance plan (ACP). New facilities may not request an ACP. -The use of non-North American stock under an approved ACP will be allowed only upon a demonstration that North American stocks are not available from any source in sufficient numbers to sustain the facility's stocking at a level equal to that during any one of the previous five years. Non-North American stocks may then be used only to the extent necessary to compensate for the difference between the number of available North American stock fish and this historic level of production. In breeding of fish used to produce offspring to be placed in net pens under an ACP, only individuals of the same stock (North American or non-North American) as determined in accordance with Appendix A may be bred with one another. No fish that are progeny of breeding between the two stocks (i.e. North American and non-North American) may be placed in the net pens. The ACP request shall contain at least the following information.
- i. In accordance with Appendix A, testing of all fish, broodstock and gametes controlled by the facility or related companies with emphasis on full characterization of fish that may be used as broodstock for spawning in 2003.

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- ii. A description of net pen stocking needs for each year that full compliance with this General Permit cannot be achieved, indicating the number of broodstock or gametes that are required to fulfill those needs. For each year class, provide the corresponding analysis from item (i), above, to explain how existing fish will be used to comply with North American stock requirements to the maximum extent possible;
  - iii. The results of a diligent search of all sources of broodstock, smolts or gametes in order to obtain North American stock. Each contact shall be documented regarding the number of broodstock, smolts or gametes available for acquisition by the facility for each year that full compliance with this General Permit cannot be achieved. In each case where acquisition may be possible, the contact must also include a statement concerning the known or believed status as to North American stock. For each year class, identify the proportion of the shortage calculated in (ii) above that will be addressed through the acquisition of North American broodstock, smolts or gametes; and
  - iv. A breeding management or acquisition plan that will be followed in order to remove all non-North American stock from the facility in the shortest possible time. This portion shall address breeding programs, as well as direct substitution of North American stock, gametes or fish in production programs for net pen stocking, and shall include sufficient information to demonstrate why the proposed time frame for achieving compliance with the use of only North American stocks cannot be achieved sooner. The plan will provide assurance that any non-North American fish utilized to meet stocking levels are kept separate as production fish and are not eligible for consideration as potential future broodstock.
- h. If a facility's ACP is approved by the Department, with or without additional conditions, the facility must comply with the approved plan and the following.
  - i. Sub-sections II.I.1.a, c and d;
  - ii. Prior to September 1 of each year, submit to the Department for review and approval, with or without additional conditions, a report documenting compliance with the approved ACP and actions proposed during the following year;
  - iii. Prior to September 1 of each year, submit to the Department a report of all actions taken to obtain gametes or fish to meet production needs for the facility during the ensuing year. The information shall be the same as described in section II.I.1.g. iii;
  - iv. As soon as possible, but in no event later than July 31, 2006, all Atlantic salmon placed in net pens must be of North American origin; and



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- v. As soon as possible, but in no event later than December 1, 2008, all non-North American Atlantic salmon must be removed from net pens.
2. Transgenic salmonids are prohibited. Transgenic salmonids are defined as species of the genera *Salmo*, *Oncorhynchus* and *Salvelinus* of the family Salmonidae and bearing, within their DNA, copies of novel genetic constructs introduced through recombinant DNA technology using genetic material derived from a species different from the recipient, and including descendants of individuals so transfected.
  3. Personnel from the Department, the Department of Marine Resources, the US Environmental Protection Agency, the National Marine Fisheries Services, the US Fish and Wildlife Service and the Maine Atlantic Salmon Commission shall be allowed to inspect the facility during normal operation hours. These personnel will provide credentials attesting to their position and will follow the site's biosecurity procedures and may, at market value, purchase random samples of salmon from the facility to monitor compliance with Special Condition II.I.1, 2 and 4. Operational records regarding compliance with this general permit shall be made available to these personnel for their inspection upon request.
  4. In accordance with the following dates, fish introduced into net pens must be marked to designate their origin so that in the event they escape or are released from the facility they may be identified. The Department will solicit comments from the Department of Marine Resources, the Maine Atlantic Salmon Commission, the US Environmental Protection Agency, the National Marine Fisheries Service and the US Fish and Wildlife Service prior to granting any approvals pursuant to this section.
    - a. In the event that a commercially reared Atlantic salmon is found in a river within the range of the distinct population segment of Atlantic salmon as defined by the US Fish and Wildlife Service and the National Marine Fisheries Service, and the facility or operating company from which it escaped cannot be identified, all facilities covered by this General Permit shall conduct third party audits of containment procedures as described in section II.I.6, below. However, the Department, in consultation with the Services, may exempt a facility from these audits when circumstances preclude the possibility that it was the source of the escaped fish. The results of audits shall be submitted to the Department within 30 days of the facility being notified of the need to conduct the audit.
    - b. After April 1 July 31, 20032004, all new fish placed in net pens must be identifiable through external means as commercially reared and be identifiable as having been stocked in Maine waters of the State. Prior to implementation of marks marking fish to be stocked, the facility shall submit to the Department for review and approval with or without additional conditions, a description of the marking method(s) to be used for this purpose.
    - c. Prior to September 1, 2003, submit to the Department for review and approval, with or without additional conditions, a report describing the methods that will be used to

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- identify the hatchery from which fish originate, as required by subparagraph (d), below. In the event similar or conflicting marking systems are proposed by different facilities, the Department may require changes be made to assure that each hatchery of origin will be uniquely identifiable.
- ed. After April 1-July 31, 2004, all new fish placed in net pens must be identifiable through external means as commercially reared and be identifiable through any means as having been stocked in Maine-waters of the State and as to which the hatchery the fish came from-which they came. Prior to implementing marks the facility shall submit to the Department for review and approval a description of the marking method(s) to be used for this purpose. In the event similar or conflicting marking systems are proposed by different facilities, the Department may require changes be made to assure that each hatchery of origin will be uniquely identifiable.
- e. Prior to September 1, 2004, submit to the Department for review and approval, with or without additional conditions, a report describing methods that will be used in the future to identify the company owning the facility, if different than the company owning the hatchery in which the fish will be reared. In the event similar or conflicting marking systems are proposed by different companies, the Department may require changes be made to assure that each company will be uniquely identifiable.
- f. After July 31, 2005, all new fish placed in net pens must be identifiable through external means as commercially reared and be identifiable through any means as having been stocked in waters of the State and as to which facility owner the fish came from.
- g. By January-September 1, 2005-2006, submit to the Department for review and approval, with or without additional conditions, a final-report describing investigations of methods or procedures that may be used to identify fish as to the facility into which they are placed. The facility shall specify for Department review and approval a description of the marking method(s) it proposes to use for this purpose. In the event similar or conflicting marking systems are proposed by different facilities, the Department may require changes be made to assure that fish will be uniquely identifiable as to the facility into which they are placed. During development of the marking system, the facility shall submit periodic progress reports describing proposals for and the results of investigations or trials. At a minimum, the progress reports shall be submitted by January 31 and July 31 of each year, or more frequently as indicated by the nature of the investigations in order to keep the Department informed of developments and proposals on a timely basis. In reviewing the report, the Department shall consider among other factors the effectiveness of company or hatchery level marking, containment management and auditing systems, the presence of escaped commercially reared fish in waters of the State and other relevant information.

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h. e.—By July 31, ~~2005~~2007, all fish placed in net pens must be identifiable through external means as commercially reared and identifiable as to the individual facility into which they were placed. Alternately, based on the report submitted pursuant to subparagraph (g) above, the Department may take the following steps regarding implementation of facility specific marking.

i. Reopen this General Permit in order to consider other or new information concerning marking; or

ii. Consider facility specific marking upon renewal of this General Permit.

5. The intentional release of Atlantic salmon to the receiving waters beyond the confines of the net pens is prohibited.
6. The facility shall employ a fully functional marine Containment Management System (CMS) designed, constructed, and operated so as to prevent the accidental or consequential escape of fish to open water. Each CMS plan shall include a site plan or schematic with specifications of that particular system. Each facility shall develop and utilize a CMS consisting of management and auditing methods to describe or address the following: site plan description, inventory control procedures, predator control procedures, escape response procedures, unusual event management, severe weather procedures and training. The CMS shall contain a facility specific list of critical control points (CCP) where escapes have been determined to potentially occur. Each CCP must address the following: the specific location, control mechanisms, critical limits, monitoring procedures, appropriate corrective actions, verification procedures that define adequate CCP monitoring, and a defined record keeping system.
  - a. The CMS will be audited at least once per year and within 30 days of a reportable escape (more than 50 fish 2 kg or larger) by a party other than the facility operator or owner qualified to conduct such audits and approved by the Department. The first annual audit shall be conducted prior to March 1, 2004. A written report of these audits shall be provided to the facility and the Department within 30 days of the audit being conducted. If deficiencies are identified during the audit, the report shall contain a corrective action plan, including a timetable for implementation and re-auditing to verify deficiencies are addressed as in the corrective action plan approved by the Department. Additional third party audits to verify correction of deficiencies shall be conducted in accordance with the corrective action plan or upon request of the Department. The facility shall notify the Department upon completion of corrective actions.
  - b. On-site at each facility personnel responsible for routine operation shall be properly trained and qualified to implement the CMS. See Special Condition II.N.
  - c. Each facility shall maintain complete records, logs, reports of internal and third party audits and documents related to the CMS. The submission of standing inventory at the facility, including all transfers in and out, losses associated with disease, predation

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or escapes reported to the Department of Marine Resources at the pen level of detail on a monthly basis according to the requirements of 12 MRSA Section 6077 shall meet the requirements of the CMS.

~~d. For existing facilities covered by this General Permit, a CMS shall be prepared and implemented on the following schedule.~~

~~By March 1, 2003, prepare an initial CMS for review and approval by the Department.~~

~~By March 1, 2004, conduct an initial audit pursuant to the CMS and submit a report, including corrective action plans and other implementation schedules as necessary, to the Department for review and approval. Any corrective action shall be completed on a schedule approved by the Department and the Department of Marine Resources.~~

de. For new facilities, a CMS shall be prepared and submitted to the Department for review and approval prior to fish being introduced into the facility.

The facility shall report any known or suspected escapes of more than 50 fish with an average weight of 2 kg each or more within 24 hours to the Department of Maine Resources at 207-624-6554 (or 800-432-7381 during off-hours).

**J. Best Management Practices for operation of the facility.**

1. Unless prohibited by prolonged periods of adverse weather, ~~The~~ facility shall remove fish carcasses from the net-pens at least once per week. However, when diseases of regulatory concern are present or suspected in the area of the facility, carcasses shall be removed more frequently in accordance with the requirements of the Department of Marine Resources or the US Department of Agriculture. Carcasses shall not be disposed of into the receiving waters, but instead shall be collected transported in leak-proof containers to an approved land-based disposal facility. Records of carcasses removed shall be maintained by the facility and made available to the Department and the Department of Marine Resources upon request.
2. The discharge of blood, viscera, or transport water containing blood associated with fish harvesting is prohibited.
3. There shall be no discharge of disinfectants, cleaning agents or similar products, except for losses that may occur incidental to the proper use of these agents. The facility shall maintain and follow best management practices for the use and control of these substances.

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4. The discharge of solid waste is prohibited. The facility shall collect used feed bags and other solid wastes for transport, recycling and/or disposal at a recycling or disposal facility approved by the Department.
5. The use of biocidal chemicals for cleaning nets on-site is prohibited. The use of air-drying, mechanical and other non-chemical procedures to control net-fouling organisms is encouraged. On-site mechanical cleaning of nets is permitted only if done in accordance with a management plan to assure that solids from these practices do not accumulate on the sea floor or cause or contribute to impairment of water quality standards, or non-compliance with section II.G. In order to control diseases of regulatory concern, net cleaning procedures required by the Department of Marine Resources or the US Department of Agriculture shall be followed. The on-shore disposal of materials removed from nets must be in compliance with applicable state and local laws. In the event that sediment monitoring indicates a potential for impact from copper or other anti-fouling agents or other established impact limits, the Department may require the use of alternate practices to avoid such effects.
6. The use of materials containing or treated with tributyl tin (TBT) compounds is prohibited.
7. When in use, horizontal predator nets shall be maintained at least 3 meters above the sea floor at all times. Nets may not impede the current flow or tidal exchange so as to contribute to the deposition of solids that would impair water quality standards. Vertical predator nets may extend to the sea floor. The storage of predator control or containment nets on the sea floor is prohibited. Any net accidentally dropped or lost during storm events that is not recovered immediately shall be tagged with a float, positioned using differential GPS, numbered, and reported to the Department within 24 hours. The net shall be recovered within 30 days from the date lost, unless the Department allows a longer time in an individual case, and the Department shall be notified on the date the net is recovered.
8. The facility shall notify the Department with written descriptions within 30 days following termination, addition to or significant reorientation of, existing mooring systems. Such changes may warrant modifications to the benthic and other monitoring plan requirements.
9. The facility shall report to the Department within 24 hours, any unusual events at the facility that might cause a significant environmental impact. Reportable “unusual events” would include, but not be limited to, fish kills (i.e. wild fish, and cultured fish beyond expected mortality rates a weekly mortality rate exceeding 150% of the average in the preceding month), -algae blooms, significant damage to nets or other equipment, interactions with marine mammals, or vessel collisions with the net pen system. Upon request by the Department, the facility shall collect and preserve a water sample, and store it until such time that the Department can retrieve it.

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**K. Husbandry Practices.** Each facility covered by this General Permit ~~that rears salmonid species~~ shall stock only a single year class of fish and fallow the site for a sufficient time to avoid the harboring or spread of diseases from one year class to the next. However, unless otherwise directed by agencies concerned with fish health, the facility may maintain fish used for broodstock purposes during the period. The carryover shall not exceed 10% of the total number of fish in the year class during the last production cycle. The facility must be in compliance with the Department of Maine Resources' rules on the importation of live marine organisms, Chapter 24 that, among other things, govern disease surveillance and reporting.

**L. ~~Best Management Practices~~ Use of drugs for disease control.**

1. ~~Only d~~Drugs approved by the FDA for finfishAtlantic salmon aquacultural purposes may be ~~discharged~~used consistent with label instructions. ~~Any approved drug not described in the facility's Notice of Intent may not be used until an amended NOI is submitted to the Department.~~ Drugs authorized, but not approved, by the FDA may be discharged consistent with II.L.3, below. All applications must comply with applicable FDA requirements. The use of vaccines as a preferred means to control disease is encouraged. The discharge of any approved drug administered as preventative measures is prohibited unless the following conditions are met: the drug must be approved by FDA ~~for the species in the pen, and~~ the treatment and route of administration must be consistent with the drug's intended use, ~~and prior approval by the Department is granted.~~ The term "discharge" includes any drug or other chemical treatment that is introduced to the fish through injection, ingestion, or immersion at the facility.
2. ~~All drugs shall be administered in accordance with the intended use, practices and concentrations prescribed on the product labels. Drugs prescribed by a Maine licensed veterinarian in an amount, frequency or application method greater than that identified on the product label shall not be discharged to the receiving water if such amounts, frequencies or application methods have not been evaluated and found acceptable by the Department relative to their effects on marine and estuarine organisms. Therefore, the discharge of any drug at a frequency or in a concentration greater than identified on the product label, or applied in a manner other than described on the product label, shall require approval by the Department prior to discharge.~~ When the need to treat or control diseases necessitates the use of a FDA approved drug not identified in a facility's NOI or inconsistent with FDA label instructions, the facility shall notify the Department as soon as becoming aware of such circumstances. If advance notice is not possible, the facility shall notify the Department on the next business day after the use has begun. The notification shall include a description of the drug, its intended purpose, amount, concentration, duration of the use and information on aquatic toxicity. In the event the use is one-time occurrence of less than 30 days, an amended NOI need not be filed. If the drug is to be used for more than 30 days, or if the use may be repeated, an amended NOI must be filed promptly pursuant to section I.D. If, upon review of information regarding the use of a drug pursuant to this section, the Department determines that significant adverse effects are likely to occur, it may restrict or limit such use.



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3. The discharge of drugs authorized by the FDA for use during studies conducted under the Investigational New Animal Drug program is prohibited unless in accordance with specific consent given in writing by the Department. Proposals for the use of investigational drugs must demonstrate that the minimum amount of drug necessary to evaluate its safety, efficacy, and possible environmental impacts will be used. Proposals must also include an environmental monitoring and evaluation program that at a minimum describes sampling strategies, analytical procedures, evaluation techniques and a timetable for completion of the program. The program must consider the possible effects on the water column, benthic conditions and organisms in or uses of the surrounding waters.
4. The discharge of any drug or other disease control chemicals shall be reported to the Department within 30 days of the application. Included in this report shall be the following: a) date and time of treatment; b) drug or disease control chemical used; c) concentration of drug or disease control chemical administered and total quantity used, including amount of feed used if applied through feed; d) approximate number of fish as well as number of pens treated; e) method of application; and f) predominant current direction during treatment.
5. The facility shall place signs at the perimeter of its leasehold to notify the public that drugs are or have been in use at that facility. The signs shall be maintained for the duration of the use and any withdrawal period following termination of use. The signs shall be at least 18 by 24 inches in size and read: "Medications are in use at this site. Contact the Maine Department of Environmental Protection or (company name) for details" and include a site designation.

**M. Best Management Practices for spill control.**

Any event that leads to the discharge of oil (including but not limited to: motor fuels, heating fuels, lubricating and hydraulic oils, waste oils, and transformer mineral oils) or hazardous substances into the waters of the State, or adjoining shorelines in a quantity sufficient to cause a film or sheen upon the water, or cause a sludge or emulsion to be deposited beneath the surface of the water or upon the adjoining shoreline shall be reported to the Department via the State Police at 1-800-4552-4664 and the National Response Center at 1-800-424-8802.

The facility shall maintain and implement a current Spill Prevention Control and Countermeasure (SPCC) Plan prepared by a Professional Engineer or other qualified professional. This plan shall be approved by the Department and include information and procedures related to the prevention of spills and unplanned discharges of petroleum products including diesel fuel, gasoline, lubrication oils, or any other hazardous materials used at the facility.

1. The plan shall provide a complete list, including quantities, of all petroleum products and other hazardous materials stored at and transferred between the facility, its support craft

and its shore-based storage facilities. The plan shall be amended when petroleum products and other hazardous materials not currently listed are transferred to the facility, and a copy sent to the Department.

2. The plan shall include descriptions of the procedures, including routine equipment inspections, used to prevent, control and/or treat spills and unplanned discharges of petroleum products and other hazardous materials according to the type and magnitude of spill or discharge.
3. The plan shall include a description of the supplies and equipment maintained onsite that prevent, control or treat spills and unplanned discharges. Supplies should include spill kits sufficient to contain a spill equal to the amount of product or material at the facility.
4. The plan shall include a description of the reporting system that will be used to alert responsible facility management, potentially effected landowners and municipalities, and appropriate legal and regulatory authorities.
5. All members of the facility's staff shall have an operation familiarity with the plan. Training shall include an annual mock spill exercise incident to review the response and reporting procedures of the plan. Documentation of staff training shall be made available to the Department upon request.
6. If the facility at any point becomes subject to the Oil Pollution Prevention regulations at 40 CFR Part 112 and stores oil in excess of the minimum threshold amounts listed in 40 CFR section 112.1(d)(2), then the SPCC Plan shall also include any additional conditions required by those regulations.

#### **N. Quality assurance for environmental monitoring and containment systems.**

Prior to any environmental data collection, infauna identification, analysis work, or containment system assessment associated with this permit, the facility shall provide to the Department documentation of the employee's or contractor's demonstrated capabilities to conduct such work. Additionally, sampling techniques and analysis methods that differ from those identified in this General Permit shall be provided to the Department for review and approval.

#### **O. Monitoring and reporting.**

Monthly Discharge Monitoring Reports required under II.E.6 and 7 of this General Permit obtained during the previous month shall be summarized for each month and reported on separate Discharge Monitoring Report Forms provided by the Department and postmarked no later than the 13th day of the month following the completed reporting period. A signed copy of the Discharge Monitoring Report Form, and all other reports required herein, shall be submitted to the State of Maine at the following address:



Aquaculture Monitoring  
Bureau of Land and Water Quality  
Maine Department of Environmental Protection  
State House Station #17  
Augusta, Maine 04333-0017

### **PART III. STANDARD CONDITIONS.**

Chapter 523, section 2 of the Department's rules also applies to discharges pursuant to this General Permit and are incorporated herein as if fully set forth.

### **PART IV. REFERENCES.**

APHA/AWWA/WPCF. 1992. Standard methods for examination of water and wastewater. 18th Ed. American Public Health Association, 1015 Fifteenth Street NW, Washington D.C. 20005. 1268 pp.

Borja, A., J. Franco, V. Perez. 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. Mar. Pol. Bul. 40: No. 12. 1100-1114.

USEPA. 1997. Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Matrices - 2<sup>nd</sup> Edition. (EPA/600/R-97/072). National Exposure Research Laboratory, Cincinnati, OH.

USEPA. 1995. QA/QC Guidance for Sampling and Analysis of Sediments, Water, and Tissues for Dredged Material Evaluations. (EPA 823-B-95-001). Office of Water, Washington, DC. Pp 50-100.

USEPA. 1986-1991. Recommended protocols for measuring selected environmental variables in Puget Sound. U.S. EPA Region 10, Puget Sound Estuary Program. Seattle, WA.

USEPA. 2000. Estuarine and Coastal Marine Waters: Bioassessment and Biocriteria Technical Guidance. (EPA 823-B-95-001). Office of Water, Washington, DC.

Wildish, D.J., H.M. Akagi, N. Hamilton, and B.T. Hargrave. 1999. A recommended method for monitoring sediments to detect organic enrichment from mariculture in the Bay of Fundy. Can. Tech. Rep. Fish. Aquat. Sci. 2286: 31p.

## Appendix A

### Atlantic Salmon Microsatellite Analysis Protocol

This protocol will be used to determine which Atlantic salmon can be used for breeding and production stock pursuant to Special Condition II.I of this General Permit. Inclusion of this protocol does not waive or diminish the objections the State has raised in State of Maine v. Norton, et al., No. 00-250-B-C (D. Me., Dec. 7, 2000). The protocol describes a standardized procedure to classify fish as either North American or non-North American stock and is largely based on the procedures used by King et al. (2001; Molecular Ecology, 10: 807-821). The facility will be responsible for providing genotype data to the US Fish and Wildlife Service and the National Marine Fisheries Service (the “Services”) for data analysis and fish classification as described herein.

#### *DNA isolation*

Genomic DNA will be isolated from tissue, fin clip or scale samples from each fish intended for use as broodstock employing either a commercially available DNA extraction, such as PureGene (Gentra Systems) or DNeasy tissue kit (Qiagen Inc.) or a phenol/chloroform based extraction system such as used in Patton et al. (1997; Can. J. Fish. Aquat. Sci., 54: 1548-1556) or, particularly for scales, a Chelex-resin based protocol such as given in King et al. (2001). Quality and quantity of DNA will be visualized on 0.8 percent agarose gels, which will include a commercially available DNA standard for quantification and size determination.

#### *Microsatellite analysis*

The loci used to classify brood fish as either North American or non-North American stock will be: Ssa85, Ssa171, Ssa197, and Ssa202 (O'Reilly et al. 1996); SSOSL311 and SSOSL438 (Slettan et al. 1995, 1996) and Ssa289 (McConnel et al. 1995).

PCR conditions for the selected loci will essentially follow that of King et al. (2001) and Patton et al. (1997) with possible minor modifications for optimization of products of individual loci. The loci will be labeled with the dyes, Ned, Hex, and 6-Fam by ABI or any other comparable commercial supplier of labeled oligonucleotides. The size standard to be used will be 400 HD Rox (ABI). Microsatellite analysis will be performed using the ABI 3100 autosequencer or any other commercial system providing equivalent results. Fragment analysis will be accomplished using a combination of GENESCAN and GENOTYPER software packages from ABI, or any other commercial system providing equivalent results. The facility will present electronic data tables from the GENOTYPER program to the Services in spreadsheet format in Excel or any other commercially available program providing equivalent results that allow the data to be easily reformatted for subsequent analyses. The output files (gel tracings) from GENESCAN and GENOTYPER will also be provided by the facility at the same time to help the Services assure data quality. Data provided must be complete at all loci for all fish.

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~~Note: In the first year of implementation (2002), only the Ssa202, SSOL311, and Ssa289 loci will be used for classifying fish as either North American or non-North American. In subsequent years all seven loci will be used.~~

*Size verification of allelic products*

To ensure accurate sizing of allelic products from the aquaculture fish relative to the designations developed in the King laboratory (see King et al. 2001), Dr. King will provide samples for use as controls. The Services will provide an adequate supply of DNA samples from representative fish of known genotypes to enable calibration of equipment throughout the term of the controlling license conditions. Control samples will be used at the inception of the study to set the automated allele designation/binning parameters of the GENOTYPER software so that all subsequent calls made for aquaculture fish will be automatically sized relative to the standards originally provided by Dr. King.

*Genetic screening*

Identification of North American aquaculture stock will be based on assignment tests performed with GeneClass,

~~[www.ensam.inra.fr/URLB](http://www.ensam.inra.fr/URLB)~~ [www.montpellier.inra.fr/URLB/geneclass/geneclass.html](http://www.montpellier.inra.fr/URLB/geneclass/geneclass.html).

Aquaculture fish will be compared to two reference groups. The first group will be comprised of samples from North America (Dennys, Ducktrap, East Machias, Machias, Narraguagus, Penobscot mainstem, Pleasant, Sheepscot, Conne, Gold, Gander, Miramichi, Saguenay, and Stewiacke rivers and aquaculture strainstocks derived from St John and Penobscot populations). The second group will be comprised of non-North American samples from at least 2 rivers each from Iceland, Norway, Finland, Scotland, Ireland, and Spain and the Landcatch aquaculture strainstock plus a hybrid strainstock crossing Landcatch with St John NB aquaculture salmon. ~~If data from additional samples become available in the future, they may be added to the reference groups to improve assignment accuracy.~~

The likelihood for assigning any given fish to each reference population will be calculated using the program GeneClass. If the ratio of the likelihood scores indicates that North American origin is at least twice as likely as non-North American origin, then that fish will be considered to be of North American origin. All other fish will be classified as non-North American stock. The Services will promptly report the results to the facility.